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Original Article

New lycosinine derivative from *Hippeastrum breviflorum*

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ABSTRACT

A new lycosinine derivative, 9-*O*-demethyllycosinine B, was isolated from the native Brazilian *Hippeastrum breviflorum* Herb., Amaryllidaceae, along with the well-known alkaloids lycosinine B and lycorine. The structure of the new compound was established by physical and spectroscopic methods. 9-*O*-demethyllycosinine B is the third lycosinine variant identified in the Amaryllidaceae family.

Introduction

The Amaryllidaceae family is one of the 20 most important alkaloid-containing plant families (Cordell, 2001). Amaryllidaceae species are able to synthesize specific isoquinoline alkaloids of eight typical skeleton types, which have demonstrated a wide range of biological activities including antitumoral, antiviral, antiparasitic, and acetylcholinesterase inhibitory activity, among others (Bastida et al., 2006; Berkov et al., 2008; Giordani et al., 2011a; McNulty et al., 2007). *Hippeastrum* is an endemic Amaryllidaceae American genus distributed from Mexico to Southern Brazil and Argentina (Dutilh et al., 2013). Although many chemical and biological evaluations of alkaloids isolated from Brazilian Amaryllidaceae plants have been carried out since 1997, most of the Brazilian *Hippeastrum* species are still unexplored.

Among the alkaloids found in *Hippeastrum* species, lycorine (**1**) and montanine are the most studied. Lycorine is a well-known antitumoral agent (Bastida et al., 2006; Ghosal et al., 1985) and has been successively isolated from *H. glaucescens*, *H. striatum*, *H. vittatum* and *H. santacatarina* (da Silva, 2005; da Silva et al., 2006; Giordani et al., 2011a; Hoffman Jr. et al., 2003). Recently, lycorine has shown to be active against the amitochondriate *Trichomonas vaginalis* Donné through a new paraptotic mechanism, which prompted the semi-synthesis of novel esterified derivatives (Giordani et al., 2011a, 2012). Montanine was isolated in an appreciable amount from *H. vittatum* and showed remarkable cytostatic and psychopharmacological effects (da Silva et al., 2006, 2008), along with the ability to inhibit the acetylcholinesterase enzyme and increase the protein phosphorylation of the MAPK signaling pathway, whose cascade of reactions is strongly related with the memory process (da Silva, 2005; Pagliosa et al., 2010). Very recently, six Amaryllidaceae alkaloids were characterized from *Hippeastrum* species for the first time (de Andrade et al., 2011, 2014; Giordani et al., 2011b).

Herein is reported the complete spectroscopic data of a new lycosinine derivative, 9-*O*-demethyllycosinine B (**2**), from *Hippeastrum breviflorum* Herbert. Lycosinine derivatives may represent a new kind of skeleton-type of Amaryllidaceae alkaloids (Ünver, 2007). The complete phytochemical procedure in *H. breviflorum* also afforded the purification of the alkaloids lycorine (**1**) and lycosinine B (**3**).

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Materials and methods

General experimental procedures

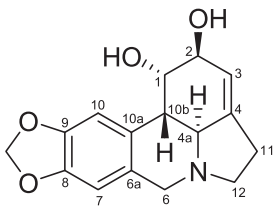
Ultraviolet (UV) spectra were determined in MeOH on an 8452-A Hewlett Packard UV-Vis spectrophotometer. ^{1}H NMR, DEPT, HMQC, HMBC and NOESY spectra were recorded in CD_3OD, on a Varian 500 spectrometer. Chemical shifts are reported in units of δ (ppm) and coupling constants (J) in Hz. EI-MS at 70 eV. HR-ESI-MS spectra were obtained on an LC/MSD-TOF (2006) Mass spectrometer (Agilent technologies). Analytical and preparative TLC was performed on silica gel plates and the spots on chromatograms were visualized by exposure under UV light (254 nm) and by Dragendorff's reagent. Silica gel Merck 60 (70–230 mesh) was used for CC and VLC.

Plant material

Hippeastrum breviflorum Herb., Amaryllidaceae, was collected in October 2012 (flowering stage) in São Francisco de Paula, in the Brazilian state of Rio Grande do Sul. Samples were identified by Julie Dutilh (Unicamp). A voucher specimen has been deposited at ICN Herbarium/UFRGS under the reference number 123123.

Extraction, purification and identification of the compounds

The fresh bulbs (3.8 kg) were crushed and macerated separately with EtOH for 48 h, three times. The combined extracts were concentrated under reduced pressure. The residue was acidified with diluted hydrochloric acid (10%) and washed with petroleum ether (4×250 ml) and then CH_2Cl_2 (3×250 ml). Both fractions were negative for alkaloids. The solution was basified to pH 8–9 with aqueous NH_4OH (25%) and extracted using CH_2Cl_2 (8×250 ml). The CH_2Cl_2 fraction from bulbs (2.15 g) was subjected to VLC on silica gel (Kieselgel – mesh 0.15/0.30, Val-de-Reuil, France) by gradient elution with CH_2Cl_2:MeOH (100:0 – A, 90:10 – B, 80:20 – C and 50:50 – D). Fraction C (0.39 g) was rechromatographed by centrifugal thin layer chromatography using CH_2Cl_2 as the starting solvent, gradually enriched with MeOH (0–50%) yielding 50 fractions (200 ml each). The fractions were monitored by TLC and 9-*O*-demethyllycosinine B (**2**, 47.1 mg) precipitated spontaneously from fraction 7. Lycorine (**1**, 4.0 mg) also precipitated from fraction 20. Fraction D (0.2 g) was chromatographed by CC starting with CH_2Cl_2 and gradually enriched with MeOH (0–50%). After semi-preparative TLC, lycosinine B (**3**, 16.2 mg) was isolated. The known compounds were identified by comparing of their physical data (IR, ^{1}H NMR, ^{13}C NMR and MS) with reference samples.



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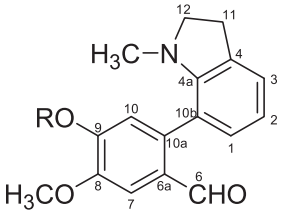

2 R=H
3 R=CH_3

Results and discussion

The alkaloid content of *H. breviflorum* has been previously evaluated through a GC–MS approach and ten alkaloids were identified (de Andrade et al., 2015). The GC–MS analysis also detected an undefined alkaloid, which after purification in the present work was isolated as the new compound 9-*O*-demethyllycosinine B (**2**). Its HRESIMS gave a mass of 284.1295, which is correct for the molecular formula $C_{17}H_{18}NO_3$ and in agreement with the theoretical mass (284.1281) of the parent $[M+H]^+$ ion. The ^{1}H NMR data for 9-*O*-demethyllycosinine B were very similar to those previously reported for lycosinine B (**3**), the presence of one aromatic methoxyl group being the only ascribable difference observed. An evident NOESY correlation between the methoxyl group and H-7 (δ 7.43) confirmed the 8-OMe group. HMBC correlation between the aldehydic carbonyl group at C-6 (δ 191.0) and H-7 was also observed, confirming a nonfused indol derivative. The remaining signals were in agreement with lycosinine B (**3**) and the NMR data for 9-*O*-demethyllycosinine B (**2**) are shown in Table 1.

Table 1
^{1}H NMR, HMQC and HMBC of compound **1**.

H	δ (J in Hz)	HMQC[a]		HMBC
1	6.84 *dt* (7.0; 1.0)	130.3 d		C-3, C-4a, C-10a
2	6.74 *t* (7.0)	118.7 d		C-4, C-10b
3	7.10 *dd* (7.0; 1.0)	124.0 d		C-1, C-4a, C-11
		120.2 s	(C-4)	
		151.1 s	(C-4a)	
6	9.45 s	191.0s		C-6a, C-7, C-8
		125.6 s	(C-6a)	
7	7.43 s	107.9 d		C-6, C-6a, C-8, C-9, C-10a
		148.5 s	(C-8)	
		155.1s	(C-9)	
10	6.74 s	117.8 d		C-6, C-6a, C-8, C-9, C-10b
		141.1 s	(C-10a)	
		131.7 s	(C-10b)	
11(2H)	2.95 *m*	28.3 t		C-3, C-4a, C-10b, C-12
12β	3.15 *dt* (9.0, 8.5)	56.9 t		C-4a, C-10b, C-11, NMe
12α	3.32 *dd* (8.5; 3.0)	56.9 t		C-4a, C-10b, C-11
OMe	3.92 s	55.1 q		C-8, C-9
NMe	2.24 s	38.5 q		C-4a, C-12

[a] Multiplicities determined by DEPT experiment.

Lycosinine A and B, previously purified from Chinese *Lycoris aurea* (L'Héritier) Herbert, are the only representatives of the lycosinine series (Yang et al., 2005) and have been considered a new skeleton-type among the Amaryllidaceae alkaloids, as well as galanthindole derivatives (Ünver, 2007). A biosynthetic route has been proposed for lycosinine compounds (Yang et al., 2005). The 9-*O*-demethyllycosinine B (**2**) is the third alkaloid from the lycosinine series to be isolated and our chemical studies on Amaryllidaceae species should prove useful in obtaining a better understanding of the biosynthetic, chemical and biological aspects of these bioactive compounds.

9-*O*-demethyllycosinine B (**2**): Amorphous solid; mp 138–140°; UV (MeOH) λ_{max} nm (logε): 243 (3.56) and 295 (3.70); ^{1}H and ^{13}C NMR: Table 1; EI-MS *m/z* (rel. int.): 283 $[M]^+$ (100), 267 (13); 254 (54), 240 (45), 222 (33), 194 (22). HR-ESI-MS $[M+H]^+$ *m/z* 284.1295 ($C_{17}H_{18}NO_3$, calcd: 284.1281).

Conflicts of interest

The authors declare no conflicts of interest.

Author's contribution

CS carried out the phytochemical process for isolation and wrote the manuscript. RBG and JPA also contributed in writing the manuscript. SB and EJO performed NMR analysis and MS helped in the collection and identification of the plant material. MVA and ATH contributed to the critical reading the manuscript. CS, RBG, JPA, SB, JASZ and JB were responsible for the spectral analysis and critical reading of the text. All authors have approved the final version for publishing.

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